Response of Commercial Cotton Cultivars to Fusarium solani

Kamel A. Abd-Elsalam^{1,2,*}, Moawad R. Omar¹, Abdel-Rheem El-Samawaty¹ and Aly A. Aly¹

¹Agricultural Research Center, Plant Pathology Research Institute, Giza, Egypt.
²Institute of Phytopathology, University of Kiel, Hermann-Rodewald-Str. 9, D-24118, Kiel, Germany (Received on April 10, 2007; Accepted on May 20, 2007)

Twenty-nine isolates of Fusarium solani, originally isolated from diseased cotton roots in Egypt, were evaluated for their ability to cause symptoms on four genetically diverse cotton cultivars. Analysis of variance showed highly significant variance among cultivars, and isolates as well as the isolate × genotype interactions were highly significant (p < 0.0001). Although most isolates showed intermediate pathogenicity, there were two groups of isolates that showed significant differences in pathogenicity on all four cultivars. None of the cultivars were found to be immune to any of the isolates. On all cultivars, there were strong significant positive correlations between dry weight and each of preemergence damping-off, survival, and plant height. Considering 75% similarity in virulence, two groups comprising a total of 29 isolates were recognized. Ninety-three percent of the isolates have the same pathogenicity patterns with consistently low pathogenicity, and narrow diversity of virulence. Isolates Fs4 and Fs5 shared the same distinct overall virulence spectrum with consistently high pathogenicity. There was no clear-cut relationship between virulence of the isolates based on reaction pattern on 4 cultivars and each of host genotype, previous crop, and geographic origin.

Keywords: Cotton, *Fusarium solani*, resistance of cultivars, symptoms, cluster analysis

Fusarium species are known to play a role in several diseases of cotton including the seedling disease complex, wilt, and boll rot (Abd-Elsalam et al., 2006). Fusarium solani (Mart.) Sacc. is one of the most ubiquitous soil fungus and a destructive plant pathogen of hundreds of hosts (Booth, 1971; Domsch et al., 1980), readily isolated pathogenic fungus from seeds and considered as more serious threat to crops, due to its close proximity to growing roots (Neergaard, 1977). The degree of losses from Fusarium root rot are indefinite, the high percentage of pathogenic isolates from soybean suggests that F. solani possibly an important pathogen (Nelson and Windels,

*Corresponding author.

Phone) 002 02 7203650, FAX) 002 02 5723146

E-mail) abdelsalamka@gmail.com

1992). Hwang et al. (1995) suggest that *Fusarium* root rot can seriously reduce yields of field pea.

F. solani is one of the organisms contributing to the seedling disease complex of Gossypium spp. (Davis et al., 1981; Johnson, 1981). Disease symptoms include seed rot, pre- and post-emergence damping-off, and seedling root rot which, individually or in combination, result in stand reductions and reduced seedling vigor that delays growth and maturity. Fusarium solani caused significant reductions in emergence of cotton and increased root discoloration of surviving seedlings (Batson and Trevathan, 1988). F. solani was isolated from cotton plants with severe foot rot in India during 1977, 1978 and 1980 (Bharathudu and Rao, 1982).

Nelson and Windels (1992), however, found F. oxysporum was the most common Fusarium species isolated from roots, but isolates were either weakly pathogenic or nonpathogenic. In contrast, nearly all isolates of F. solani were highly pathogenic (Nelson et al., 1997). In cotton, F. oxysporum were frequently isolated from cotton seedlings infected with damping-off (Aly et al., 1996; El-Samawaty, 1999). Eighty-three percentage of F. solani isolates collected from cotton were pathogenic (Abd-Elsalam et al., 2006). Batson and Borazjani (1984) indicated that the percentage of emergence of seedlings from fumigated soil artificially infected with F. solani was significantly lower than that from noninfected soil. The F. solani isolates were more virulent than each of *F. equiseti*, *F. moniliforme* and *F.* graminearum collected from cotton in Louisiana (Colyer, 1988).

Development of host plant resistance requires that genes for resistance to a specific pathogen be accumulated from advanced germplasm stocks or identified in more distant germplasm and transferred into superior lines (Stanton et al., 1994). Some improvement in resistance to cotton seedling disease pathogens in the Multi-Adversity Resistance germplasm pools has been reported (Thaxton et al., 1991). The interaction between *F. solani* isolate and soybean cultivar was done to evaluate commercial soybean cultivars commonly grown in southern Minnesota (Nelson et al., 1997). Hwang et al. (1995) evaluated the resistance of pea cultivars to *F. solani*.

Current knowledge of sources and stability of resistance

Table 1. Isolates of *Fusarium solani* analyzed in this study

Isolates code	Geographic origin	Previous Crops	Host cultivar
Fs1	Assuit	Egyptian clover	Giza 83
Fs2	Assuit	Egyptian clover	Giza 83
Fs3	Sohag	Egyptian clover	Giza 83
Fs4	Assuit	Egyptian clover	Giza 83
Fs5	Assuit	Egyptian clover	Giza 83
Fs6	Assuit	Egyptian clover	Giza 83
Fs7	Assuit	Egyptian clover	Giza 83
Fs8	Sohag	Egyptian clover	Giza 83
Fs9	Sohag	Egyptian clover	Giza 83
Fs10	Assuit	Egyptian clover	Giza 83
Fs11	Sohag	Egyptian clover	Giza 83
Fs12	Sohag	Egyptian clover	Giza 83
Fs13	Assuit	Egyptian clover	Giza 83
Fs14	Sohag	Egyptian clover	Giza 83
Fs15	Beheira	Egyptian clover	Giza 70
Fs16	Gharbiya	Onion	Giza 86
Fs17	Minufiya	Egyptian clover	Giza 89
Fs18	Daqahliya	Egyptian clover	Giza 86
Fs19	Daqahliya	Pea	Giza 86
Fs20	Dumyat	Egyptian clover	Giza 45
Fs21	Dumyat	Faba-Bean	Giza 45
Fs22	Dumyat	Egyptian clover	Giza 45
Fs23	Beheira	Egyptian clover	Giza 70
Fs24	Daqahliya	Egyptian clover	Giza 86
Fs25	Dumyat	Faba-Bean	Giza 45
Fs26	Minufiya	Faba-Bean	Giza 89
Fs27	Minufiya	Faba-Bean	Giza 89
Fs28	Beheira	Egyptian clover	Giza 70
Fs29	Minufiya	Onion	Giza 89

to *Fusarium* root rot in cotton field is limited because no cultivars have recently been evaluated in Egypt. A clear understanding of the extent of variation in virulence would be helpful in devolving cotton cultivars with stable resistance. Therefore, this study was undertaken to evaluate resistance to *F. solani* in currently available some commercial cotton cultivars used for planting and in breeding program in Egypt. And, also to examine the pathogenic variability among the isolates of *F. solani* originating from different cotton-growing areas of Egypt.

Materials and Methods

Fungal cultures. Fungal isolated from cotton roots were purified by single-spore and identified. *Fusarium* isolates grown on PDA were identified according to Nelson et al. (1983). Twenty-nine isolates of *F. solani* collected from cotton fields were used in this study (Table 1). Isolates were stored in 25% (v/v) glycerol at -70°C and prior to use

subcultured onto potato dextrose agar (PDA) and incubated at 25°C.

Host materials. Four commercial cotton cultivars (Giza80, Giza 86, Giza 89 and Giza 90) were used to study phenotypic variation for resistance of *F. solani* isolates in the seedling stage. The basis for cultivar selection in this study is based on their adaptation to climatic variability and geographic distribution.

Preparation of grain inoculum. For inoculation, autoclaved clay loam soil was infested with the mixture of isolates at the rate of 10 g/kg of soil. Three to five 4-mm-diameter agar discs of each isolate were placed in (1 L) screw-top jars, which had been half-filled with moist sorghum grain (100 g grain + 100 ml water) and autoclaved twice for 30 min. The inoculated jars were incubated at room temperature in natural light for three weeks to ensure complete colonization of the grain. After incubation, infested grain was removed from the jars, air-dried in a laminar-flow microbial transfer hood for two days, and stored at 4°C until needed. Infested soil was dispensed in 10-cm-diameter clay pots and these were planted with 10 seeds per pot.

Greenhouse evaluations. The pathogenicity of the F. solani isolates was determined by inoculating cotton cultivars. Twenty-nine isolates of F. solani, obtained from different locations (Table 1) were used in the current research. Batches of autoclaved clay loam soil were separately infested with inoculum of each isolate at a rate of 50 g/kg of soil. Infested soil was dispensed in 10-cmdiameter clay pots and these were planted with 10 seeds per pot for each of the tested cultivars (Giza80, Giza 86, Giza, 89, and Giza 90). Control plants were grown in autoclaved soil. The climate control for the heating system for these tests ranged from 30.5 ± 3.5 to 40 ± 4 °C. There were three pots (replicates) for each treatment. Treatments and controls were replicated twice. Preemergence damping-off was recorded 15 days after planting. Other pathogenicity parameters were recorded 45 days after planting.

Statistical analyses. The experimental design was a randomized complete block with three replicates. Data from pathogenicity tests were subjected to analyses of variance (ANOVA). Cotton genotypes and fungal isolate were used as variables. Least significant difference (LSD) was used to compare cultivar means. Statistical calculations were done with the software package, STATISTICA 6 (StatSoft Inc., Tulsa, Oklahoma, USA). Cluster analysis of data from all pathogenicity parameters, using the UPGMA method, was conducted to generate a dendrogram showing relationships

between isolates with regard to their virulence to the four cultivars using the SPSS 6.0. software.

Results

Greenhouse pathogenicity tests. ANOVA of the effect of isolate, cultivar and their interaction on F. solani rot root resistance in four cotton cultivars is shown in Table 2. Analysis of variance showed significant influences of isolates and cultivars on all five measures. However, ANOVA revealed a significant isolate x cultivar interaction. Therefore, differences in pathogenicity were assessed on each cultivar separately (Figs. 1-5). This indicates that neither the cotton cultivar component alone, nor the F. solani isolate component alone, is sufficient to explain the differences observed in Fusarium rot root incidence in cotton seedlings. Due to the very highly significant effect of cultivar × isolate interaction on preemergence damping-off, a least significant difference (LSD) was calculated to compare isolate means within each cultivar (data not shown). These comparisons showed that the differences in preemergence damping-off between isolates and the control were not the same for each cultivar, that is, cultivars responded differently to the isolates. Isolates Fs4 and Fs5 were highly pathogenic on all the cultivars (Fig. 1). All cultivars were susceptible to all isolates and thus were considered universally susceptible (Figs. 2 and 3). The cultivars Giza 86 and Giza89 were moderately susceptible (Fig. 4). For any cultivar, the number of isolates, which significantly reduced plant height, was much greater than that of the isolates, which significantly reduced dry weight (Figs. 4 and 5). Based on preemergence damping-off, postemergence damping-off, plant survival, dry weight and plant height, most of the commercial Pima cultivars (G. barbadense L.) were susceptible to F. solani. The four

Table 2. Analysis of variance of interactions between host cultivars and isolates of *Fusarium solani* under greenhouse conditions

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Parameter and Source of variation y	D.F	M.S	F.value	P > F				
Preemergence damping-off								
Replication	2	65.888	1.0580	0.3488				
Cultivar (C)	3	257.720	4.1384	0.0070				
Isolate (I)	29	1509.421	24.2379	0.0000				
$C \times I$	87	230.542	3.7020	0.0000				
Error	238	62.275						
Postemergence damping-off								
Replication	2	150.263	0.9647					
Cultivar (C)	3	5734.650	36.8153	0.0000				
Isolate (I)	29	987.316	6.3384	0.0000				
$C \times I$	87	287.811	1.8477	0.0001				
Error	238	155.768						
Survival								
Replication	2	6.972	0.3738					
Cultivar (C)	3	4698.967	26.6278	0.0000				
Isolate (I)	29	2275.791	12.8963	0.0000				
$C \times I$	87	278.266	1.5769	0.0037				
Error	238	176.469						
Plant height								
Replication	2	42.904	1.2827					
Cultivar (C)	3	229.647	6.8656	0.2792				
Isolate (I)	29	340.903	10.1918	0.0002				
$C \times I$	87	45.037	1.3465	0.0000				
Error	238	33.449		0.0409				
Dry weight								
Replication	2	1584.953	0.5521					
Cultivar (C)	3	320669.800	111.5262	0.0000				
Isolate (I)	29	48613.602	16.9074	0.0000				
C×I	87	6576.852	2.2874	0.0000				
Error	238	2875.286						

yReplication is random, while each of cultivar and isolate is fixed

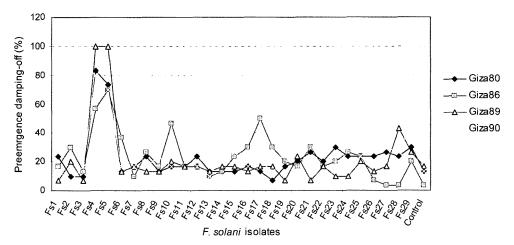


Fig. 1. Effect of interaction between cotton cultivars and *F. solani* isolates on preemrgence damping-off of cotton seedlings under greenhouse conditions.

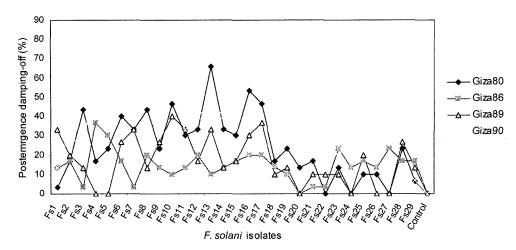


Fig. 2. Effect of interaction between cotton cultivars and *F. solani* isolates on postemrgence damping-off of cotton seedlings under greenhouse conditions.

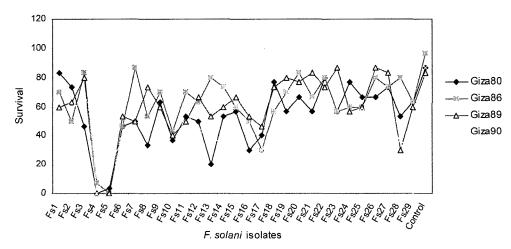


Fig. 3. Effect of interaction between cotton cultivars and F. solani isolates on survival of cotton seedlings under greenhouse conditions.

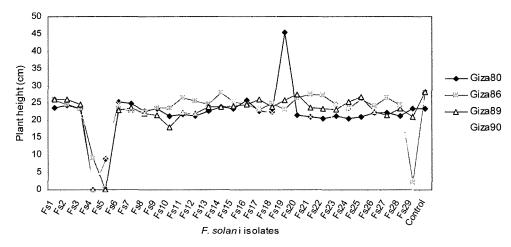


Fig. 4. Effect of interaction between cotton cultivars and F. solani isolates on plant height of cotton seedlings under greenhouse conditions.

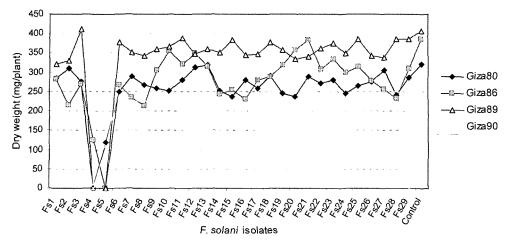


Fig. 5. Effect of interaction between cotton cultivars and F. solani isolates on dry weight of cotton seedlings under greenhouse conditions.

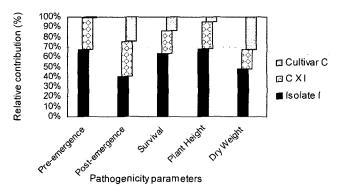


Fig. 6. Relative contribution of cotton cultivars *F. solani* isolate and their interaction to variation in pathogenicity parameters of cotton seedlings.

cultivars were affected to varying degrees by *F. solani* in greenhouses. Data regarding the relative contribution of cultivar, isolate and cultivar × isolate to variation in diseases incidence are shown in Fig. 6. Isolate was the most important source of variation in plant height. Whilst, cultivar was the most important source of variation in dry weight. The interaction was the most important source of variation as it accounted for 35% of the explained variation in postemergence damping-off.

Correlation between pathogencity parameters. Correlations among variables used for evaluating pathogenicity of *F. solani* isolate are shown in Table 3. In Giza 80, there was a strong significant positive correlation between preemergence damping-off and survival, plant height, and dry weight; however, there were no significant correlations between preemergence and postemergence damping-off. A highly significant positive correlation was observed among all variables used for evaluating pathogenicity on Giza 86. No significant correlation was found between survival and each of preemergence damping-off and postemergence

damping-off of Giza 80, Giza 89, and Giza 90.

Virulence patterns. Dendrogram of *F. solani* isolates based on the category of their interaction with four cotton cultivars are shown in Fig. 7. Dissimilarity distance (DD) between isolates in the dendrogram that was generated following cluster analysis ranged from 3 to 25% (Fig 7). The dendrogram represented two main clusters, major cluster and minor cluster (DD=3). Minor cluster comprised only two isolates (Fs4 and Fs5). Major cluster could be divided into two groups, I and II. Group I included two isolates (Fs10 and Fs 29). Group II included all the rest of isolates at the genetic similarity of 98%. Among 29 isolates, 27 had similar virulence patterns, isolates Fs4 and Fs5 showed a highly virulence reaction to the four cultivars. The dendrogram showed no relationship among the clustered isolates and their geographical origin.

Discussion

The information concerning behavior of plant and pathogen, disease resistance and pathogenicity, is crucial in plant breeding as well as in selection of cultivars. To the best of our knowledge, this is the first published report to investigate variation in virulence in *F. solani* populations towards commercial cotton cultivars. Pathogenicity of 29 isolates of *F. solani* was tested on four cotton cultivars under greenhouse conditions. Preemergence damping-off, postemergence damping-off, survival, plant height and dry weight were used as criteria to evaluate pathogenicity.

The ANOVA has been advocated as a means of detecting specificity in the host-pathogen relationship, when all the isolates of the pathogen can cause disease on all the test cultivars of the host (Vanderplank, 1968; Vanderplank, 1982; Vanderplank, 1984). However, it might not be a

Table 3. Correlation among variables used for evaluating pathogeneicity of *F. solani* isolates on seedlings of cotton cultivars under greenhouse conditions

Cultivar	Variable	2	3	4	5
Giza 80	1. Preemergence damping-off (%)	0.227	0.609**	0.744**	0.832**
	2. Postemergence damping-off (%)		0.630**	0.145	0.078
	3. Survival (%)			0.457*	0.604**
	4. Plant height (cm)				0.654**
	5. Dry weight (mg/plant)				
Giza 86	1. Preemergence damping-off (%)	0.495**	0.938**	0.728**	0.508**
	2. Postemergence damping-off (%)		0.763**	0.602**	0.626**
	3. Survival (%)			0.771**	0.622**
	4. Plant height (cm)				0.831**
	5. Dry weight (mg/plant)				
Giza89	1. Preemergence damping-off (%)	0.321	0.833**	0.916**	0.904**
	2. Postemergence damping-off (%)		0.177	0.306	.0319
	3. Survival (%)			0.754**	0.751**
	4. Plant height (cm)				0.929**
	5. Dry weight (mg/plant)				
Giza 90	1. Preemergence damping-off (%)	0.290	0.611**	0.722**	0.613**
	2. Postemergence damping-off (%)		0.808**	0.507**	0.564**
	3. Survival (%)			0.660**	0.564**
	4. Plant height (cm)				0.857**
	5. Dry weight (mg/plant)				

ylinear correlation coefficient (r) is significant at p < 0.05 or p < 0.01

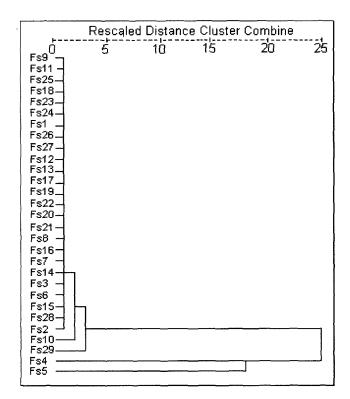


Fig. 7. Dendrogram showing differences in virulence of 29 isolates of *F. solani* based on their ability to infect four different cotton cultivars.

sensitive test as environment may cause significant interaction between host cultivars and pathogen isolates (Kulkarni and Chopra, 1982).

ANOVA showed that the main effects of both cultivars (p = 0.000) and isolates (p = 0.000) were very highly significant source of variation in all the tested parameters as was cultivar \times isolate interaction (p = 0.000). Statistically significant test result among the different cultivar, isolate, and the interaction between cotton cultivars and isolates suggest that physiologic specialization exists within F. solani isolate pathogenic to cotton. It also implies that the resistance of the tested cultivars is a mixture of both vertical and horizontal resistance and there are significant differences among cultivars in both types of resistance. Similarly, pathogenicity of the tested isolates is also a mixture of virulence and aggressiveness, and the isolates significantly differ in both types of pathogenicity. Van Eeuwijk et al. (1995) obtained similar results and concluded that resistance is horizontal (i.e., race-non-specific).

The resistance response may be influenced by many variables. Distinct differences occurred between cultivars in the length of time for symptom development and in the frequency of diseased plants, indicating different degrees of susceptibility. The differences in plant responses at seedling and adult plant growth stages reported in the literature of Populer (1978) make designing an effective breeding strate-

gy a challenge. Varying levels of resistance among cultivars is probably also related to some inherent physiological characteristic of a particular cultivar and the ability of a particular pathogen isolate to overcome that resistance. Variability in response of cultivars to isolates may arise from differences in environmental conditions (Douiyssi et al., 1998).

The genetic basis for this delayed symptom development or partial resistance needs to be studied in order to take advantage of these findings for breeding more resistant cultivars. Incorporation of resistance to *F. solani* into breeding efforts could significantly improve control measures and potentially reduce fungicide use.

Cluster analysis has been used previously to determine the similarity in virulence of pathogen isolates (Lebeda and Jendrulek, 1987; Schilder and Bergstrom, 1990; Sah and Fehrmann, 1992). Very slight differences in cluster compositions were observed for repeated data when two experiments were analyzed separately. No association was observed between the virulence patterns and the isolates location. In conclusion, the diversity of virulence in the population of F. solani collected from cotton growing areas in Egypt is narrow. Isolates Fs4 and Fs5 were closely related pathogenically. All cultivars tested were considered susceptible to F. solani since a percentage of all cultivars became infected. Origin of host is not an important determinant for the virulence of F. solani on cotton. These results indicate that isolates of F. solani cause root rot on cotton and the cultivars grown in cotton producing areas in Egypt lack resistance to this pathogen. These results also may help growers select cultivars that are less susceptible to F. solani, which should aid in the management of this disease. Supplementary research are planned to include a wider range of cultivars and pathogen isolates, to assess the resistance of all commercial cotton cultivars to these diseases.

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